THE SYNTHESIS OF LIGNIN-LIKE COMPLEXES BY FUNGI

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The ability of some of the common soil fungi to synthesize lignin-like complexes when grown on a sucrose-nitrate medium was demonstrated by Thom and Phillips (8). The black and brown species, commonly found growing on decaying vegetation at the soil surface, contained up to 29 per cent of these lignin-like substances, whereas the colorless or light-colored fungi contained little lignin. Likewise, some of the brown and black wood-destroying bracket fungi contained as much as 54 per cent of these materials, whereas the light-colored species contained only about 2 to 4 per cent. In a later paper Phillips (5) reported a chemical study of the lignin-like material in one of these bracket fungi. This material was found to resemble lignin in some of its chemical reactions but contained almost no methoxyl groups. Although Thom and Phillips (8) pointed out the possible relationship of these lignin-like substances in the filamentous fungi to the formation of soil organic matter, no further work along these lines has been reported. The present paper presents results obtained with 12 filamentous fungi, mostly species that are found in the soil or on decaying plant materials. Both pigmented and unpigmented species were selected.

METHODS

The fungi were grown in 3-liter wide-mouth Erlenmeyer flasks stoppered with cotton and containing 500 ml. of nutrient medium.

The compositions of the media used, apart from the nitrogen source, were as follows:

A. Czapek's medium: K_2HPO_4, 1.0 gm.; KCl, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; $FeSO_4 \cdot 7H_2O$, 0.01 gm.; sucrose, 30 gm.; and distilled H_2O, 1 liter.

B. Same as A except NaCl, 0.2 gm., instead of KCl, 0.5 gm., and trace elements instead of $FeSO_4 \cdot 7H_2O$, 0.01 gm.; plus $CaSO_4 \cdot 2H_2O$, 0.1 gm.

C. Same as B plus $CaCO_3$, 10 gm.

D. Same as B except K_3PO_4, 1 gm., and K_2HPO_4, 0.5 gm., instead of K_2HPO_4, 1 gm.

The trace elements added to media B, C, and D were supplied at the rate of 10 ml. per liter of a solution having the following composition: $FeSO_4 \cdot 7H_2O$, 150 mgm.; $ZnSO_4 \cdot 7H_2O$, 125 mgm.; $CuSO_4 \cdot 5H_2O$, 30 mgm.; $MnCl_2 \cdot 4H_2O$, 25 mgm.; $NaMoO_4 \cdot 2H_2O$, 4.5 mgm.; and distilled H_2O, 1 liter.

The media were sterilized in the autoclave, inoculated in duplicate or triplicate with pure cultures, and incubated at 25°C. for 1 month.

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Prior to deciding upon the temperature of incubation to be used, a preliminary experiment was run with four cultures grown at both 25° and 30°C. The rates of growth of *Cladosporium fulvum* 5668, *Cladosporium* 5464.2, and *Alternaria* were about equally good at the two temperatures, whereas *Helminthosporium* 5428.16 grew somewhat better at the lower temperature. The weight of fungus obtained per unit of sugar consumed was about 20 per cent higher at the lower temperature.

At the end of the incubation period the pH values of the media were determined colorimetrically and the sugar contents by the method of Shaffer and Hartmann (7). The fungus mat was dried at 40 to 45°C. for about 5 days to constant weight. This method was used to avoid decomposition, which was observed to take place at 105°C., and also to minimize errors in the determination (3) of lignin.

Lignin-like material was determined by the 72 per cent sulfuric acid method (6), and the corrected value was obtained by subtracting the protein (nitrogen $\times$ 6.25). The protein correction in this particular study is only an approximation, since fungi may contain varying amounts of nonprotein nitrogenous substances. The ash content of the lignin fractions of three of the fungi varied only from 0.5 to 0.76 per cent; hence this determination was not made on the remainder of the samples. The slight correction was ignored in reporting the results.

EXPERIMENTAL RESULTS

The pertinent data concerning the growth and carbohydrate consumption of the various species of fungi are given in table 1 and the analyses of the fungus mats in table 2. All of the data in the two tables are average values of either duplicate or triplicate cultures.

In general, all of the organisms studied made fairly good growths under the conditions of culture, provided the reaction of the medium did not become appreciably acid. The growths obtained in the moderately acid media were lower than expected. Although these fungi tolerated acid media it is quite apparent that they did not thrive under these conditions. In most cases where the media were kept at near neutrality the sugar was virtually all consumed during the 30-day growth period.

All three sources of nitrogen were utilized by the six cultures of fungi used in the comparative tests. In most cases sodium nitrate gave better results than ammonium sulfate or ammonium nitrate, chiefly because it kept the pH at a higher level. Where pH was not a factor there were usually no marked differences between the three nitrogen sources; in fact, with some of the organisms ammonia nitrogen gave higher yields than did nitrate.

The most satisfactory of the four media was C, containing trace elements and adequate $CaCO_3$ to keep the reaction above the neutral point. It is evident that the trace elements, and calcium, favored growth, although there was no critical comparison of growth with and without these elements in media otherwise identical.

TABLE 1

Growth and efficiency of sugar utilization of several fungi grown in different media
Incubated 30 days at 25°C.

FUNGUS	MEDIUM USED	NITROGEN SOURCE*	pH OF MEDIUM	WEIGHT OF FUNGUS	SUGAR CONSUMED	ECONOMIC COEFFICIENT†	CARBON ASSIMILATION‡
				gm.	*gm.*	*per cent*	*per cent*
Cladosporium 5464.2	A	$NaNO_3$	8.0	5.097	15.00	34.0	42.4
	B	$NaNO_3$	8.2	5.207	15.00	34.7	43.2
	B	$(NH_4)_2SO_4$	2.4	1.606	6.05	26.6	33.2
	B	NH_4NO_3	2.4	1.920	7.25	26.5	33.1
	C	$NaNO_3$	8.4	5.027	15.00	33.6	41.9
	C	$(NH_4)_2SO_4$	7.3	5.869	14.76	39.7	49.6
	C	NH_4NO_3	7.6	5.392	14.84	36.4	45.4
	D	$NaNO_3$	7.4	3.034	11.12	27.3	34.1
	D	$(NH_4)_2SO_4$	5.4	0.398	1.75	22.9	28.6
	D	NH_4NO_3	5.4	0.647	2.73	23.8	29.7
Cladosporium fulvum 5668	A	$NaNO_3$	6.4	1.421	8.11	17.5	20.7
	B	$NaNO_3$	8.1	5.033	15.00	33.5	39.6
	B	$(NH_4)_2SO_4$	2.0	2.249	10.40	21.3	25.2
	B	NH_4NO_3	6.8	5.305	14.89	35.6	42.0
	C	$NaNO_3$	7.8	5.344	14.80	36.1	42.6
	C	$(NH_4)_2SO_4$	7.2	5.875	14.75	39.8	47.0
	C	NH_4NO_3	7.6	5.586	15.00	37.2	43.9
	D	$NaNO_3$	8.2	5.293	15.00	35.3	41.7
	D	$(NH_4)_2SO_4$	4.4	2.126	10.49	20.2	23.9
	D	NH_4NO_3	7.2	5.568	14.59	38.1	45.0
Helminthosporium 5428.16	A	$NaNO_3$	7.9	3.123	15.00	20.8	24.4
	B	$NaNO_3$	8.2	4.543	15.00	30.2	35.4
	B	$(NH_4)_2SO_4$	2.3	0.970	4.41	21.9	25.6
	B	NH_4NO_3	2.3	1.010	4.96	20.3	23.8
	C	$NaNO_3$	8.6	4.179	15.00	27.9	32.7
	C	$(NH_4)_2SO_4$	7.6	5.016	15.00	33.4	39.1
	C	NH_4NO_3	8.0	5.184	15.00	34.6	40.5
	D	$NaNO_3$	8.4	4.423	15.00	29.5	34.5
	D	$(NH_4)_2SO_4$	4.5	1.470	6.70	21.9	25.6
	D	NH_4NO_3	7.2	4.960	15.00	33.1	38.8
Alternaria	A	$NaNO_3$	7.5	1.505	7.58	19.7	21.7
	B	$NaNO_3$	7.7	2.892	13.19	21.9	24.1
	B	$(NH_4)_2SO_4$	2.4	0.481	3.14	15.3	16.8
	B	NH_4NO_3	3.0	1.548	5.78	26.8	29.5
	C	$NaNO_3$	7.7	3.438	14.16	24.2	26.6
	C	$(NH_4)_2SO_4$	7.9	0.607	3.68	17.0	18.7
	C	NH_4NO_3	7.9	3.778	14.73	25.7	28.3
	D	$NaNO_3$	7.6	2.823	13.62	20.6	22.7
	D	$(NH_4)_2SO_4$	2.4	0.678	3.48	19.5	21.5
	D	NH_4NO_3	6.0	2.632	9.50	27.8	30.6

TABLE 1—*Concluded*

FUNGUS	MEDIUM USED	NITROGEN SOURCE*	pH OF MEDIUM	WEIGHT OF FUNGUS	SUGAR CONSUMED	ECONOMIC COEFFICIENT	CARBON ASSIMILATION
				gm.	*gm.*	*per cent*	*per cent*
Gliocladium fimbriatum 459.5128	A	$NaNO_3$	6.6	1.175	15.00	7.8	9.4
	B	$NaNO_3$	8.4	4.727	15.00	31.5	37.8
	B	$(NH_4)_2SO_4$	2.1	1.715	6.05	28.3	34.0
	B	NH_4NO_3	7.6	4.549	15.00	30.4	36.5
	C	$NaNO_3$	8.4	4.380	15.00	29.2	35.0
	C	$(NH_4)_2SO_4$	7.5	5.206	15.00	34.7	41.6
	C	NH_4NO_3	7.8	4.549	15.00	30.4	36.5
	D	$NaNO_3$	8.4	4.866	15.00	32.5	39.0
	D	$(NH_4)_2SO_4$	4.6	3.043	11.63	26.4	31.7
	D	NH_4NO_3	7.7	4.320	15.00	28.8	34.6
Metarrhizium	A	$NaNO_3$	6.6	0.299	9.89	3.0	3.2
	B	$NaNO_3$	8.3	3.289	15.00	21.9	23.7
	B	$(NH_4)_2SO_4$	2.4	0.927	5.92	15.7	17.0
	B	NH_4NO_3	7.1	3.085	15.00	20.6	22.3
	C	$NaNO_3$	7.4	4.310	15.00	28.7	31.0
	C	$(NH_4)_2SO_4$	7.7	2.750	15.00	18.4	19.9
	C	NH_4NO_3	8.4	3.625	15.00	24.2	26.1
	D	$NaNO_3$	8.6	3.590	15.00	24.0	25.9
	D	$(NH_4)_2SO_4$	2.4	1.075	5.81	18.6	20.1
	D	NH_4NO_3	7.6	3.153	15.00	21.0	22.7
Dematium pullulans 5695.354D	B	$NaNO_3$	7.9	3.555	14.80	24.0	25.2
Aspergillus giganteus 5–5581.13A	B	$NaNO_3$	8.2	3.534	14.80	23.9	24.1
Aspergillus niger 142	B	$NaNO_3$	7.4	3.672	15.00	24.5	24.7
Gliocladium 454–4640.428	B	$NaNO_3$	8.6	4.713	15.00	31.4	39.6
Helminthosporium 27.07	B	$NaNO_3$	8.6	5.528	15.00	36.9	38.4
Humicola 5239	B	$NaNO_3$	8.2	3.524	15.00	23.5	26.3

* Nitrogen was added at the rate of 3.0 gm. per liter of sodium nitrate or its equivalent.
† Weight of fungus divided by weight of sugar consumed.
‡ Weight of carbon in fungus divided by weight of carbon in sugar utilized.

The efficiency of use of the sugar supplied is commonly expressed in terms of weight of fungus produced divided by weight of sugar consumed (economic coefficient), or weight of carbon in the fungus divided by weight of carbon in the sugar used. The latter value is usually somewhat higher than the former because the percentage of carbon in the fungus is commonly higher than that in sugar. Both values are given in table 1.

Under the best conditions, the percentage of the carbon in the sugar that was converted into cell material was approximately 40 to 50 for *Cladosporium*, *Helminthosporium*, and *Gliocladium*, and 25 to 30 for *Alternaria*, *Metarrhizium*, *Dematium*, *Aspergillus*, and *Humicola*. These data are in good agreement with values of 30 to 50 per cent reported by Waksman (10, p. 244). In almost all cases where

the growth conditions were poor and a considerable portion of the added sugar was not consumed, less fungal material was formed per unit of sugar consumed than under good growth conditions. Apparently this observation holds for most microorganisms kept under ordinary atmospheric conditions.

The percentage of carbon in the 12 cultures of fungi, given in table 2, varied from 42.7 to 53.1. This compares with a value of 42.1 for sucrose. The nitrogen content varied from 2.4 to 4.4 per cent. This value would, of course, be expected to vary markedly with the quantity of nitrogen supplied. The C:N ratios averaged 15.3 with variations from 10.7 to 22.4. These values are not very much higher than those of the majority of the soils in the northern half of the United States.

The content of crude lignin varied from 2.8 to 30.9 per cent for the various organisms studied. This crude lignin consists of lignin-like complexes, ash, and

TABLE 2

Composition of the fungi studied

FUNGUS	CARBON	NITROGEN	C:N RATIO	CRUDE LIGNIN	PROTEIN IN CRUDE LIGNIN	LIGNIN	SUGAR CONVERTED TO LIGNIN
	per cent	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
Cladosporium 5464.2	52.52	2.41	21.8	30.90	6.93	23.97	12.0
Cladosporium fulvum 5668	49.75	2.56	19.4	25.05	6.85	18.20	8.9
Helminthosporium 5428.16	49.40	3.71	13.3	24.85	8.25	16.60	7.3
Helminthosporium 27.07	43.92	3.21	13.7	27.65	6.15	21.50	11.5
Aspergillus giganteus, 5–5581.13A	42.68	3.83	11.1	9.70	3.35	6.35	2.2
Aspergillus niger 142	42.70	2.66	16.1	9.50	3.10	6.40	2.3
Gliocladium fimbriatum 459.5128	50.42	2.90	17.4	3.79	0.93	2.86	1.3
Gliocladium 454–4640.428	53.10	2.37	22.4	2.78	0.80	1.98	0.9
Alternaria	46.18	4.03	11.5	9.20	2.40	6.80	2.2
Dematium pullulans 5695.354D	44.07	3.59	12.3	10.58	3.48	7.10	2.5
Metarrhizium	45.65	3.24	14.1	5.45	1.88	3.57	1.1
Humicola 5239	47.02	4.39	10.7	12.78	4.65	8.13	2.8

some unknown organic nitrogenous materials. The quantities of these last-named materials, expressed as proteins, are shown in the table. By subtracting these values from those given for crude lignin, the percentages of lignin, or lignin-like materials, are obtained. The methoxyl contents of the lignin-like materials in *Cladosporium fulvum* 5668, *Cladosporium* 5464.2, and *Helminthosporium* 5428.16 were 0.65, 1.03, and 0.32 per cent respectively. These values are much lower than those commonly reported for the lignins of higher plants and are in agreement with the findings of Phillips (5).

The last column in table 2 shows the percentage of the carbon in the sugar assimilated that was converted into lignin-like materials. Phillips' figure of 61 per cent carbon in the lignin of fungi was used in making these calculations.

These data confirm the conclusions of Thom and Phillips that the black or brown species of fungi, so far as tested, are able to synthesize considerable quan-

tities of lignin-like materials, whereas the light-colored species synthesize smaller amounts or none. According to the results in table 2 all species synthesized some of these materials, but of course the method used includes any material that resists the sulfuric acid treatment.

DISCUSSION

These studies are of interest from the standpoint of practical agriculture, since they show that certain types of soil fungi, if supplied with adequate mineral nutrients including nitrogen, can convert an energy source, such as sugar, into lignin-like materials. These crude lignin-like materials have C:N ratios and certain other properties similar to those of soil humus. Most soil fungi can utilize a wide variety of sources of energy; even cellulose is converted into lignin by some of the organisms used in the present studies. Work along these lines is now in progress.

Thom and Phillips point out that the black and brown molds thrive on decaying vegetation found at or just above the soil surface and not on the same materials after being plowed under. This fact is of interest in connection with the present tendency to emphasize the merits of keeping the crop residues on the surface, as in stubble mulch farming. In the process of decomposition, whether at the surface or on the plow sole, much of the lignin in the plant residues would resist decomposition and be converted into humus. In addition, if lignin-synthesizing organisms are active, considerable humus should also be formed from the sugar, cellulose, and other readily available carbon sources. It is well, however, not to overemphasize this point until we have practical information to support the laboratory results and indications.

The rate and the completeness of decomposition of fungal tissues of various species have been considered by a number of investigators. The conclusions of the earlier workers differ markedly, as pointed out by Jensen (2). Most of these workers observed that both bacterial and fungal cells decomposed rather readily although a few mentioned the formation of resistant humus-like residues. In Jensen's work no clear influence of C:N ratio of the microbial substance on decomposition was observed. On the other hand, Heck (1) and Norman (4) found a very clear correlation between the C:N ratio of the fungus materials and the rate of decomposition. In many cases Jensen obtained colloidal, brown to black, humus-like compounds as residues from the decomposition of fungi. Waksman (9), likewise, reported the synthesis of similar substances by mixed cultures using cellulose as an energy source. In contrast, Norman says that he found no evidence for the existence of a very resistant residue from fungal tissue. The present studies show that these differences among workers can be explained in large part by the composition of the organisms used. Apparently neither Norman nor Heck used species of fungi that we would now expect to be synthesizers of large quantities of lignin-like compounds. On the other hand, at least one of Jensen's species (*Stachybotrys* sp.) probably possesses this ability. The mycelia of *Cladosporium* and *Helminthosporium*, used in the present study, would probably decompose as did Jensen's *Stachybotrys*, whereas *Gliocladium* would be expected to behave as did the organisms used by Heck and by Norman.

SUMMARY

The synthesis of lignin-like complexes by 12 cultures of filamentous fungi grown on a mineral-sucrose medium was studied. The mycelium of *Cladosporium* contained as high as 24.0 per cent of these lignin-like substances. The average values for the genera studied were *Cladosporium* 21.1, *Helminthosporium* 19.0, *Humicola* 8.1, *Dematium* 7.1, *Alternaria* 6.8, *Aspergillus* 6.4, *Metarrhizium* 3.6, and *Gliocladium* 2.4. These values represent the nonnitrogenous portion of the fungal substance that resisted digestion with 72 per cent sulfuric acid. In general, the black or brown fungi were comparatively high in lignin complexes, whereas the colorless or light-colored organisms contained lower percentages. The failure of previous workers, using various genera, to agree as to the ability of fungi to form substances that are resistant to decay is largely explained by these results.

Under the best growth conditions, that is at pH 7 or slightly above and with adequate trace elements present, approximately 40 to 50 per cent of the carbon of the sucrose was converted into cell material by *Cladosporium*, *Helminthosporium*, and *Gliocladium*; the corresponding values for the other organisms were 25 to 30 per cent. The C:N ratios of the fungus material varied between 10.7 and 22.4 per cent with an average value of 15.3.

It is pointed out that the high-lignin organisms grow mostly on decaying vegetation at or above the soil surface. A system, such as that used in stubble mulch or trash mulch farming, would therefore be expected to be favorable to a high yield of humus.

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